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Tuning the Emission Properties of a Fluorescent Polymer using a Polymer Microarray Approach – Identification of an Optothermo Responsive Polymer

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Polymer microarrays were prepared using inkjet printing mixtures of acrylate monomers each with a common fluorescent fluorene co-polymer. Fluorescent analysis of each of the features on the array allowed identification of polymers that could tune the fluorescence under a variety of insults. The “hit” polymers were made into beads via reverse suspension polymerization their fluorescence properties analyzed.

Conjugated fluorescent polymers,¹ have attracted huge amounts of interest because of their application in light emitting diodes,² optoelectronic devices³ and biosensors,⁴ in part due to their tunable electrical and optical properties while possessing attractive mechanical properties and processing characteristics.⁵

Since the electrons are conjugated along the backbone, the properties of the conjugated fluorescent polymer dyes are highly sensitive to minor external structural perturbations and local electron density changes that occur upon binding to other molecules.⁶ It has been demonstrated that the chiral conformation of a polythiophene derivative can be manipulated by a “wrapping polymer” and its colour controlled,⁷ while the fluorescence of conjugated polymers, such as polythiophene derivatives or fluorophores such as rhodamine, when grafted onto thermo-sensitive polymers can be switched on and off by altering the temperature.⁸ Recently, conjugated fluorescent polymers have become candidates to replace molecular dyes and quantum dots for various fluorescence based biomedical-imaging applications,⁹ while being used as fluorescent enhancers.

Compared to molecular dyes, conjugated fluorescent polymers have advantages in terms of brightness of emission, high extinction coefficients and good photo-stabilities,¹⁰ which make them suitable for various fluorescence imaging tasks. In addition, conjugated fluorescent polymers are biologically compatible with lower toxicities¹¹ than quantum dots.¹² Another approach to generate highly intense fluorescent signals is to incorporate them into particles, thus polymer beads loaded with fluorophores are widely used in many biomedical applications, including as standards and calibrants, and for cell tracking and labeling.¹³ Such fluorescent polymer beads thus provide a powerful platform for visualizing biological structures from the anatomical to the cellular and the monitoring of dynamic physiological processes. Here polymers used for manipulating fluorescent polymers were identified using a high-throughput approach, with polymer microarrays applied to the identification of polymer targets that could control the properties of conjugated polymers with monomers chosen according to the literature.^{7,8a} The polymer microarrays were constructed from monomer collections to produce large numbers of polymers on a glass slide by inkjet printing of monomers, cross-linkers and photo-initiators and subsequent *in situ* polymerization initiated by UV light. This technique enables the polymer composition and the ratio of each monomer to be easily varied, resulting in arrays with large numbers of polymer features.¹⁴ Such polymer microarrays have previously been used for the rapid identification of synthetic polymers with specific biological functions,¹⁵ with the interactions between cells and hundreds to thousands of individual polymers being simultaneously probed.^{16,17} The interaction between conjugated fluorescent polymers and a matrix of polyacrylates/acrylamides was thus envisaged as a means of tuning and controlling the fluorescence emission of the polymer, with a library approach allowing much greater chemical space to be explored than has hitherto been possible. Here polymer microarray technology was used for the discovery of polymers that would not only promote the optical stabilization of conjugated fluorescent polymers, but

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also allow the properties of the dye to be tuned, physically and thermally. Eleven acrylates and acrylamides were used for microarray preparation (Table 1, ESI Methods)

Table 1 Monomers used for polymer microarray preparation*

Labels.	Abbreviation	Monomers
A	HEMA	2-Hydroxyethyl methacrylate
B	EGDMA	Ethylene glycol dimethacrylate
C	DMAEMA	Dimethylaminoethyl methacrylate
D	DMOBAA	Diacetone acrylamide
E	NIPAA	N-Isopropyl acrylamide
F	CHMA	Cyclohexyl methacrylate
G	DMC	Methacrylateoethyltrimethyl ammonium chloride
H	CEA	2-Carboxyethyl acrylate
I	DEAA	N,N-Diethylacrylamide
J	AAM	Acrylamide
K	HPOAA	2-Hydroxy-3-phenoxypropyl acrylate

*: Polymers are coded by the monomer composition and their ratios. For instance, the copolymer prepared from 15 drops of HEMA and 5 drops of EGMA is coded as A15B5

The fabrication of polymer microarrays was achieved using an inkjet printing approach as has been described in detail elsewhere.¹⁸ During fabrication of the array a solution of the dye (0.005 wt%) was added to each polymer (Fig. 1, Fig. S1 and S3, ESI[†]) which was generated from solutions of the two monomers, the cross-linker N,N'-methylene-bis(acrylamide) (MBA) solution and the photoinitiator 1-hydroxycyclohexyl phenyl ketone. Across each line of the microarray two monomer solutions were printed in varying ratios designed as 20/0, 15/5, 10/10, 5/15, 0/20 (based on the number of drops of monomers printed) with 4 replicates for each ratio, with a common level of cross-linker (12.7 wt%) for each polymer. The glass slides were exposed to UV light (365 nm) for 30 min to initiate *in situ* polymerization after printing.

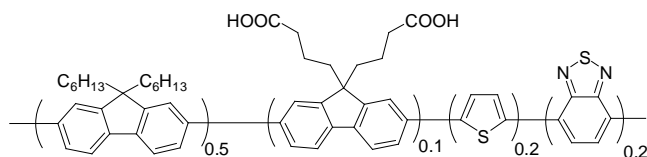


Fig. 1 The structure of the conjugated fluorescent polymer used in this study, which was synthesized using Suzuki cross-coupling chemistries.

Following microarray fabrication the array was screened, with fluorescent images of each polymer feature captured using an inverted fluorescent microscope (excitation 350–370 nm) with a 20× objective (Fig. S2, ESI[†]). When the conjugated fluorescent polymers were immobilized within the polymers, the colour of polymers became cyan in colour indicating that the polymers interacted with the dye and affected its fluorescent emission. The fluorescent intensity of each spot was analyzed and compared to the starting conjugated fluorescent polymer allowing nine polymers to be identified that either increased or

decreased the fluorescent intensities, as well as those that appeared to totally quench the dye's fluorescence.

Fluorescent images of each polymer spot on the array (20×55 spots in total) were analyzed with Image J (Table S1, ESI[†]).¹⁸ The relative fluorescent intensity was used for comparison among the features and was calculated using following equation (1):

$$I_r = \frac{I_{fp} - I_p}{I_f} \quad (1)$$

Where, I_{fp} is the absolute fluorescent intensity of a polymer spot with the immobilized conjugated fluorescent polymer, I_p is the auto-fluorescence intensity of the polymer spot without the dye; I_f is the fluorescent intensity of the dye.

Thus the relative fluorescent intensities of 1100 polymer spots were calculated and are given in descending order (Fig. 2a). Three polymer candidates that increased the relative fluorescent intensity and three candidates where the fluorescent intensities were reduced after immobilization and three that appeared to have quenched or lost the dye were chosen for the printing of the secondary arrays for further confirmation (Fig. 2b). These 9 polymers were used for the preparation of a secondary array with the fabrication of 20 replicates for each of the polymers (Table 2). The printing process was the same as above. Features with a negative I_r represented host polymers that had suppressed the dye's fluorescence and were not studied further.

Table 2 Polymer candidates used for preparation of the secondary microarray

I_r	$I_r > 1$	$0 < I_r < 1$	$I_r \approx 0$
Polymer candidates	A10G10 A5B15 E15G5	E5G15 E5H15 B15J5	F15I5 F5I15 A15K5

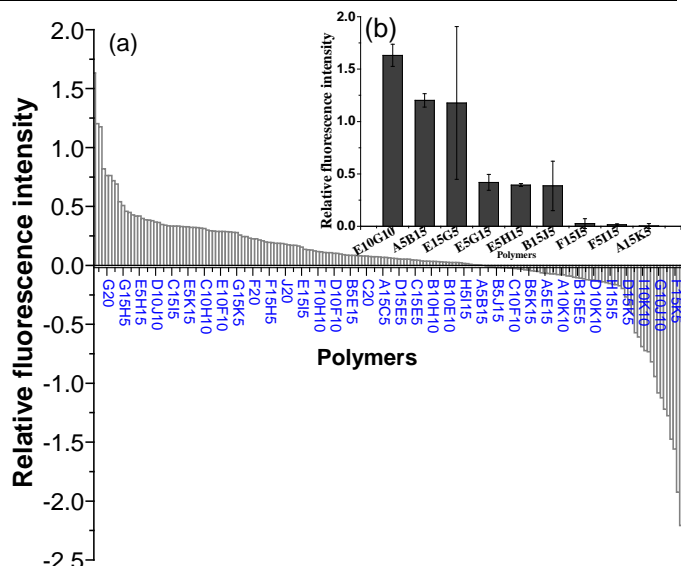


Fig. 2 Fluorescent intensity analysis of the polymer microarray:

(a) The relative fluorescent intensities of the spots on the primary polymer microarray (data are means of quadruplicate)

polymers of each combination). STDEVs were calculated (see SI) but are not shown here for clarity); (b) The insert shows the 9 polymers taken forward, with three polymers showing increased fluorescent intensity; three with reduced fluorescence intensity and three with no fluorescence (The error bars are STDEV, $n=4$).

The fluorescent images of the spots of the secondary array were analyzed (Fig. 3) and showed good agreement with the primary array screen.

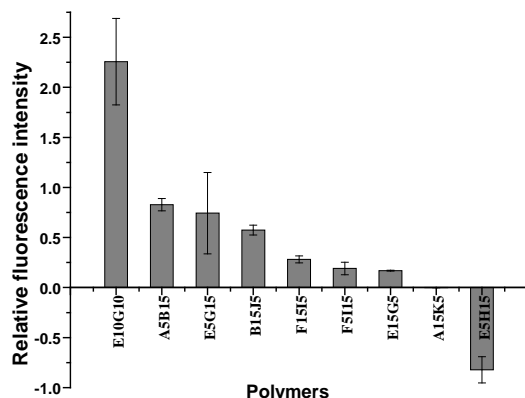


Fig. 3 The fluorescent intensities of the polymers as fabricated on the secondary array. The fluorescent images of the polymer features were captured and the fluorescent intensities analyzed using Image J and equation (1). (The error bars are STDEV, $n=20$)

This array was screened and analyzed and 5 combinations were identified to take forward for further examination. The three polymer combinations that enhanced the fluorescent intensity of the conjugated fluorescent polymer (E10G10, A5B15 and E5G15) and another two E15G5 and A15K5, that had no or limited fluorescence were up-scaled to produce polymer beads with the entrapped conjugated fluorescent polymer (Scheme S1, Fig. S4, ESI[†], Methods). The fluorescent beads were characterized at different temperatures (25°C, 35°C, 45°C and 55°C) and compared to solutions of the conjugated fluorescent polymers.

From the fluorescent images, the beads are fluorescent corresponding to the emission light of the dye with wavelength of 500–700 nm when $\lambda_{\text{Ex}} = 450$ nm (Fig. 4). Quantitative fluorescent analysis showed that the fluorescent intensity of A5B15-A was some 2.5 times larger than that of the conjugated fluorescent polymer, while the fluorescent intensities of E5G15-A and E15G5-A were similar to that of the dye. A15K5-A was only the third of the fluorescent intensity of the pure dye. The fluorescence emission peaks of the immobilized dye shifted 20–40 nm to shorter wavelengths. It indicates that the main chains of the embedded-conjugated fluorescent polymer may have been twisted or strained to interrupt their conjugation during the polymerization process, resulting in a blue shift for the emission wavelength.¹⁹

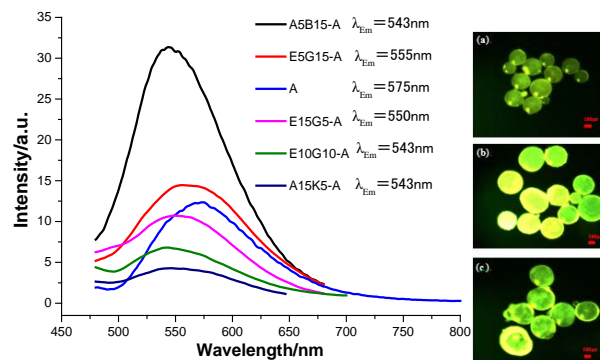


Fig. 4 Left) The fluorescent intensity of the fluorescent conjugated polymer (A) and polymer beads with the immobilized dye, A5B15-A, E5G15-A, E15G5-A, E10G10-A and A15K5-A respectively. The fluorescent dye and beads were suspended in (PEG-400:H₂O=2:1) and analyzed at 25°C with $\lambda_{\text{Ex}} = 450$ nm. The concentration of the dye was estimated according to the dye immobilized in the beads during fabrication (see Fig. S5, ESI[†]). Right) Fluorescent images of the polymer beads. (a), (b) and (c) polymer beads A15K5-A, A5B15-A and E15G5-A respectively.

The influence of temperature on the fluorescence intensity of the conjugated fluorescent polymer was investigated and showed negligible effects on the intensity of the emission peak as the temperature increased from 25 to 60°C (Fig. S6, ESI[†]). However, the fluorescent intensity reduced when the dye was immobilized in the polymers A5B15, E15G5 (Fig. S7, S8, ESI[†]) and it dropped dramatically when the conjugated fluorescent polymer was embedded in the polymer E5G15 with the fluorescence intensity dropping from 14.5 to 6.5 RFU when the temperature increased from 25 to 60°C (Fig. 5), which was recovered when cooled down to 25°C again. Thus E5G15-polymer beads exhibited thermo-fluorescence - fluorescence that could be switched on and off by just by altering the temperature over many cycles (Fig. 6).

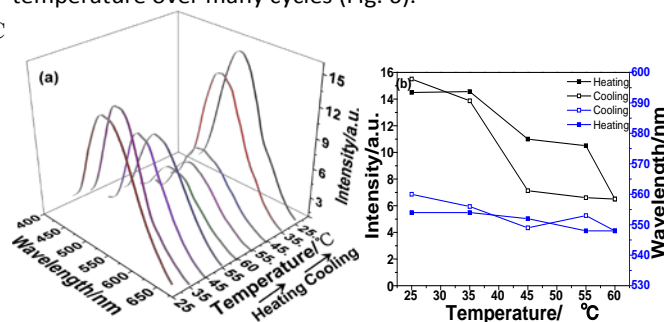


Fig. 5 Fluorescence intensity change in polymer beads made from E5G15-A following the temperature rise from 25 to 60°C and then cooling to 25°C measured with an $\lambda_{\text{Ex}} = 450$ nm (a) and (b) corresponding fluorescence intensities and emission wavelengths at various temperatures.

The temperature dependence of the intensity of the fluorescent beads could be the result of the 'on/off' switching of the aggregation-caused quenching (ACQ)²⁰ of the dye immobilized in the host polymer under different temperatures

(Video S1 and S2, ESI[†]) because the shift of the emission peak was very small, less than 10 nm in wavelength (Fig 5b). PNIPAA is a well-known thermo-responsive polymer, therefore the host polymer consisting of monomer NIPAA has the possibility to trigger the fluorescence switching of beads by polymer shrinking at high temperature and swelling at room temperature. The temperature-dependent thickness alteration of the host polymer was examined using a rheometer as the temperature changing from 10 to 55 °C and cooled down to 10 °C again under constant compressive forces (2 or 4 kPa). During the measurement, an oscillatory shear stress was imposed on the polymer at a frequency of 1 Hz to obtain a corresponding oscillatory shear strain. Extrapolation to zero compressive force showed that the relative thickness of the hydrogel polymers reduced when they were heated from 10 to 55 °C, shrinking some 4% in thickness and increased for 1–2% when the hydrogels were cooled down to 10 °C again (Fig. 6b, c).

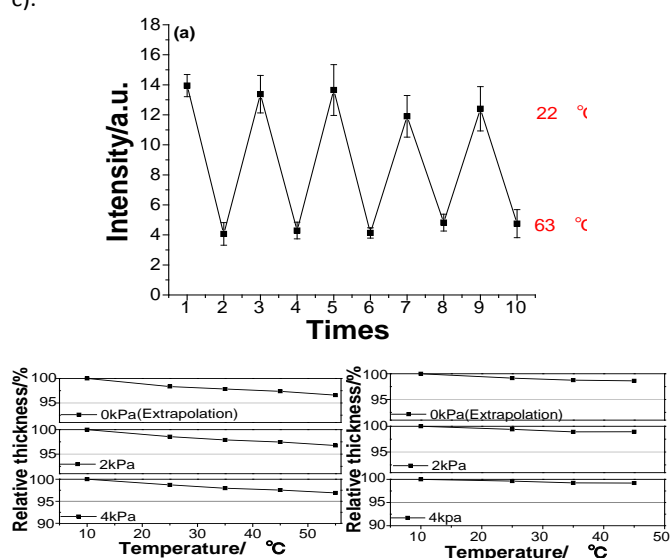


Fig. 6 (a) Switching cycles of the fluorescence intensity of the E5G15-A polymer beads in PEG400/H₂O (2/1) triggered with temperature changes between 22 and 63 °C ($\lambda_{\text{ex}} = 450$ nm) (error bars are STDEV, $n=5$). (b) Change in relative thickness of the E5G15 polymer as a function of temperature in response to actual (2 and 4 kPa) and extrapolated (0 kPa) compressive forces as the temperature was raised from 10 to 55 °C and (c) the temperature reduced from 55 to 10 °C.

In conclusion polymer microarrays were fabricated with polymers immobilizing a fluorescent polymer. Upon screening of the arrays, it was found that copolymers of specific combinations (of acrylates and acrylamides) could affect the fluorescence of the dye. For example the polymer HEMA/EGDMA (1/3) enhanced the fluorescence of the dye 2.5 fold while polymer HEMA/HPOAA (3/1) reduced its fluorescence to 1/3. Host polymer NIPAA/DMC(1/3) was thermo-responsive, with switch-on and off of the fluorescence of the immobilized dye because of ACQ. The work demonstrates that the microarray approach based on

fluorescent polymers could identify host polymers, manipulating the fluorescence of conjugated polymers for various applications such as labeling and imaging of tissues, smart sensors, switching devices, molecular logic gates and other electronic device.

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